

Review Article



The Medical Uses of Hydrogen



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Correspondence:

Myung-Hee Chung, MD, PhD

Department of Pharmacology, Seoul National University, 103 Daehak-ro, Jongno-gu, Seoul 03080, Korea.

E-mail: mhchung@snu.ac.kr

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ORCID iDs

Myung-Hee Chung 

<https://orcid.org/0000-0001-8105-5369>

Jai Youl Ro 

<https://orcid.org/0000-0002-5559-143X>

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Myung-Hee Chung ^{1,2} Jai Youl Ro ³

¹Department of Pharmacology, Seoul National University College of Medicine, Seoul, Korea

²Neuroscience Research Institute, Gachon University, Incheon, Korea

³Department of Pharmacology, School of Medicine, Sungkyunkwan University Natural Sciences Campus, Suwon, Korea

ABSTRACT

Hydrogen (H₂) has been the focus of industrial interest as a pollution-free fuel. In addition, hydrogen is also a hot topic in medicine. Recently, animal and human experiments have shown that hydrogen is effective in treating various diseases, raising the possibility of developing hydrogen as a new therapeutic agent. The therapeutic effect of hydrogen is due to its antioxidant action to remove reactive oxygen species (ROS) effectively. Currently, it is known that ROS is involved in almost all diseases that afflict mankind. The therapeutic effect of hydrogen was reported first by Ohsawa and colleagues in 2007. They induced brain injury in rats by ischemia-reperfusion and found that inhalation of hydrogen gas (2%) markedly suppressed brain injury by inhibiting the actions of ROS. Since then, many researchers have found that hydrogen was effective in the ROS-involved diseases (diabetes, kidney complication of renal dialysis, acute brain infarction, inflammatory diseases of skin, muscle and joint, side effects of radiotherapy, skin wrinkles and post-exercise fatigue) in animal models and patients. In particular, hydrogen gas, unlike conventional antioxidants, 1) easily passes through the cell membrane and thus, is easily distributed to all organs and tissues of our body, 2) uniquely removes the most toxic hydroxyl radicals (•OH) of ROS, and 3) has no adverse or toxic effects with doses (0.4 and 1.6 ppm) used in all experiments reported. Based on the results of these experiments, attempts to use hydrogen as a therapeutic are actively underway. Given the fact that ROS are constantly produced in our bodies, the use of hydrogen gas or hydrogenated water is thought to help maintain health through treatment or prevention of diseases.

Keywords: Hydrogen; Oxidative stress; Anti-aging; Antioxidants

THE CHEMICAL PROPERTIES AND INDUSTRIAL USES OF HYDROGEN

The colorless, tasteless and odorless gas, hydrogen (H₂) was discovered by Henry Cavendish in England in 1766. Hydrogen is produced into water when burned by oxygen (O₂) (2H₂+O₂ → 2H₂O). From this phenomenon, the word hydrogen is derived from the Greek words “hydro” meaning water “gennao” meaning production. Hydrogen (the element symbol: H, the atomic weight: 1.00749 g/mol, one-sixteenth of oxygen; the atomic weight of oxygen: 15.999 g/mol), is the lightest element on Earth. Hydrogen atoms combine with each other to form a gaseous

hydrogen molecule (H_2). Hydrogen gas is highly reactive and easily explodes in contact with air or oxygen. If more than 4% of hydrogen is mixed in the air, it is ignited and exploded by heat, fine static electricity, light, high temperatures without external stimuli.¹ Hydrogen was once used to fly airships and balloons but was replaced by helium because of its high reactivity. Due to its reactivity, hydrogen is used to form ammonia (NH_3) with nitrogen (N_2), methyl alcohol (CH_3OH) with carbon monoxide (CO) and hydrochloric acid (HCl) with chlorine (Cl_2), to produce saturated solid lipid such as margarine from unsaturated liquid lipid and to remove sulfur from the petroleum.²

Recently, it has been in the spotlight as a pollution-free fuel for cars and power plants. This is because hydrogen gas has the highest combustion heat, 143 kJ/g (pure carbon, methane gas, and gasoline: 33, 64, and 47 kJ/g, respectively) but produces only water as a final product. For practical use, however, there are many difficulties to overcome. First, hydrogen exists in its natural state as a hydrogen compound, such as hydrocarbons, rather than hydrogen gas, so lots of energy is needed to get hydrogen from the sources. Second, hydrogen is a gas that takes up a lot of volume so a hydrogen container is needed that can catch a large volume of hydrogen into a small volume. Although 850 times the volume of hydrogen can be absorbed into metals such as palladium,³ but the problem is that palladium is expensive. Therefore, development of metals or alloys to trap hydrogen with cheap and large storage capacity is required.

THE FIRST EXPERIMENT ON POSSIBILITY OF HYDROGEN FOR MEDICAL USE

The medical efficacy of hydrogen was first reported by the Ohsawa group⁴ in 2007. They observed that hydrogen selectively removes hydroxyl radicals ($\bullet OH$) and only removes superoxide radicals ($\bullet O_2^-$) and hydrogen peroxides (H_2O_2) when they are at high concentrations. Of reactive oxygen species (ROS), $\bullet OH$ is the most destructive to cells and $\bullet O_2^-$ and H_2O_2 act as cell signaling mediators at lower concentrations, so eliminating $\bullet O_2^-$ and H_2O_2 may rather impair normal physiological functions of cells. Therefore, selective removal of $\bullet OH$ suggests that hydrogen is a very desirable antioxidant. In the same experiment, they also observed that inhalation of hydrogen gas (2%) to the rats inhibited tissue damage and function impairment of brains significantly that were injured by ischemia-reperfusion known as a situation in which ROS are produced.

OTHER EXPERIMENTS ON THE HYDROGEN EFFECTS IN CELLS, ANIMALS AND HUMANS

After the Ohsawa group's report,⁴ many researchers have tried to prove the effectiveness of hydrogen through experiments on cells, animals, and humans. These experiments support the potential effectiveness of hydrogen for medical use.

Effectiveness of hydrogen in cellular experiments

Ohsawa's group⁴ showed the action of hydrogen to remove ROS using PC12 cells (cancer cells of adrenal medulla). When cultured in the presence of antimycin A, PC12 cells died due to destruction of lipids in the cell membranes. But the same experiment done in the culture containing hydrogen, hydrogen reduced the cell death remarkably by removing $\bullet OH$. Antimycin A is known to induce the production of ROS in the mitochondria by interfering

with electronic flow in the mitochondrial electron transport chain. The auditory hair cell was also damaged by ROS produced by antimycin A. But when the same experiment was conducted in a solution containing hydrogen, the damage was significantly reduced.⁵ This experiment was conducted based on the assumption that ROS is involved in the hearing impairment by noise.⁶ Lymphocytes and small intestinal cells in the culture media containing hydrogen were also protected from the impaired cell division and apoptosis by irradiation.⁷ Human skin fibroblasts can be damaged in the culture media with high concentration of glucose because ROS are produced by increased metabolism. But the cell damage was significantly reduced in the experiment using hydrogen-containing culture media.⁸ Some researchers⁹ suggested that hydrogen may have even an anti-cancer effect since they observed that the growth of human tongue cancer cells was slower than normal cells when the cells were treated with hydrogen. Itoh et al.¹⁰ reported that hydrogen suppresses FcεRI-mediated signal transduction and prevents degranulation of mast cells, implying that hydrogen is presumably effective on allergic diseases.

Effectiveness of hydrogen in animal experiments

The effectiveness of hydrogen was also observed in animals in which various ROS-involved diseases were induced experimentally or in the animals possessing such genetic diseases. The following are the results of the animal experiments.

Diseases of central nervous system

1. Stroke

Stroke is a disease in which blood vessels in the brain are blocked or burst, causing the drop of blood supply to brain tissue, which is called ischemia (leading to hypoxia). If ischemia persists, the ischemic area is completely damaged (called cerebral infarction) and the patient dies. To save the patient, blood should be supplied to the ischemic area. Ironically, however, when blood is fed back (reperfusion) to the ischemic region, ROS are produced in the ischemic region, causing tissue damage. This is referred to as 'the production of ROS by ischemia-reperfusion',^{11,12} and the removal of ROS produced at this time is an important part of the treatment.

Researchers observed the effectiveness of hydrogen on the cerebral infarction in mice or rats induced by ischemia-reperfusion. Usually, ischemia-refusion is induced by ligating arteries for a certain period of time and then releasing them. When administering hydrogen to these rats by inhalation⁴ or intraperitoneal injection,^{13,14} they observed significant improvements in weight, motor activities, body temperature and the smaller size of the cerebral infarction in the treated animals. Different methods were employed to induce ischemia, which were surgical injury,¹⁵ traumatic injury^{16,17} or cerebral hypothermia.¹⁸ In the brain injuries induced by these methods, treatment with hydrogen reduced injury size and recovery period.

2. Cognitive disorder and dementia

One of the serious diseases of the brain is cognitive impairment (poor learning ability and decreased memory) that is a typical symptom of dementia. Cognitive impairment was induced experimentally in young mice by trapping them in a small wire cage, i.e., giving physical restraint. Using this condition, generation of ROS inhibit the development of the areas responsible for cognitive function.¹⁹ However, supplying hydrogen water (HW) to the mice under physical restraint prevented the learning impairment and memory loss.²⁰ Alzheimer's disease is known to be caused by the accumulation of fragments of amyloid beta protein in brain cells, which produce ROS and damage brain cells.²¹ The intraperitoneal

administration of hydrogen saline (HS) to the mice given amyloid beta protein fragments also reduced oxidative stress and inflammation, and improved memory impairment.²²

3. Parkinson's disease

Parkinson's disease is a motor disorder caused by impaired function of dopaminergic neuron of substantia nigra. It is known that ROS are also involved in the functional impairment of these neurons. To test the possibility that hydrogen has effectiveness on Parkinson's disease, researchers observed the effect of hydrogen on the destruction of dopaminergic neuron of substantia nigra and motor dysfunction caused by 6-hydroxydopamine²³ or MPTP(1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine),²⁴ which are used for preparing animal model of Parkinson's disease by producing ROS. The results showed that hydrogen was inhibitory on the neuron destruction and progress of motor dysfunction.

4. Spinal cord injury

Hydrogen was also tested on spinal cord injury. Chen et al.²⁵ induced the cord injury by contusion and administered HS peritoneally. They found that in hydrogen-treated animals, inflammation, apoptosis and oxidative stress were decreased whereas the production of brain-derived neurotrophic factor (BDNF) was increased. These results suggest that hydrogen promotes the recovery of spinal cord injury.

Cardiovascular diseases

1. Myocardial infarction

Among heart diseases, an acute life-threatening disease is myocardial infarction caused by no blood supply to myocardial muscle due to blocking of coronary arteries. It is also a disease caused by ischemic-reperfusion as in stroke, and at this time, active oxygen is produced, damaging the heart muscle. Hayashida et al.²⁶ administered hydrogen into rats by inhalation during the induction of the myocardial infarction by tying and untying the coronary artery entering the left ventricle and found that the production of ROS in the infarct area and infarct size were reduced compared to the control animals.

2. Arteriosclerosis

Arteriosclerosis is a representative disease involving ROS. In this disease, wastes such as cholesterol, oxidized lipids and leukocytes accumulate under the endothelium creating a mass called atheroma. In the process of the atheroma formation (atherosclerosis), inflammatory reaction occurs and thus ROS acts as an accelerating factor in the mass formation.²⁷ To test the effect of hydrogen on arteriosclerosis, high fat diets alone or high fat diets with HW were given to mice genetically prone to arteriosclerosis and compared the progress of atherogenesis. The result showed that the arteriosclerosis was inhibited significantly in the hydrogen-treated group.²⁸

Liver diseases

As in the heart, researchers^{29,30} tested the effects of hydrogen inhalation on the liver damage induced by ischemic-reperfusion and observed that the liver damages were inhibited significantly. Hydrogen also showed the protective effect on hepatitis induced by concanavalin A.³¹ This chemical has an inflammation-inducing action by stimulating neutrophils to produce ROS.^{32,33}

Kidney diseases

The anti-cancer drug called cisplatin is an antineoplastic agent frequently used for various

solid tumors. However, its use is restricted due to the toxicity on the kidney. The toxicity on the kidney turns out to be due to ROS production.³⁴ Based upon this fact, researchers³⁵ tested the effect of hydrogen inhalation on the cisplatin-induced kidney toxicity in mice and found that nephrotoxicity was alleviated without affecting its anti-cancer activity.

Lung diseases

The effectiveness of hydrogen was also tested for lung diseases caused by ROS. Deterioration of lung function and lung tissue damage during lung transplantation is a typical example of lung injury by ischemic-reperfusion. Therefore, Saito et al.³⁶ observed the improvement in the lung compliance, oxygenation level, and other lung functions after being transplanted by placing the isolated lung in a hydrogen-melted preservation solution before transplantation. Hydrogen was also protective oxygen toxicity by the observation that injection of saturated HS reduced lung injury caused by exposure to hyperbaric oxygen.³⁷ Chronic obstructive pulmonary disease (COPD) is a progressive pulmonary disease caused by harmful gases or particles. Coadministration of hydrogen and cigarette smoke to rats slowed the development of COPD-like lung disease.³⁸

Intestinal diseases

The most painful bowel disease is chronic ulcerative colitis. To verify the effectiveness of hydrogen on this colon disease, mice were administered dextran sulfate sodium to induce colitis and then allowed to drink hydrogenated water. It was found that the inflammatory reactions were suppressed.³⁹ Among the difficulties suffered by colon cancer patients are side effects such as diarrhea and bleeding after radiation. ROS are known to be involved in these side effects. When hydrogen was administered, the colon damages by irradiation were significantly inhibited.⁴⁰ Use of hydrogen can be a useful tool to mitigate the side effects of irradiation on colon cancer patients.

Pancreatic disease

Pancreatitis is also a very painful condition among many inflammatory diseases. Experimentally, pancreatitis was induced in 2 ways, by shock using high air pressure⁴¹ or by administering the amino acid L-arginine.⁴² In the former experiment, the effect was observed by injecting HS saline into the abdominal cavity, in the latter, into the vein. Hydrogen has been found to suppress the progression of pancreatitis.

Eye disease

Glaucoma is a painful and serious eye disease that causes vision loss. The space between the lens and the cornea (anterior chamber) is filled with water (aqueous humor) which can enter this space. After filling it up, the water leaves this space through a thin tube and enters the vein. If there is an abnormality in the continuous circulation of this water, the intraocular pressure may rise, causing visual impairment. This condition is called glaucoma. If the increase of pressure rise is severe, the retina is pressed and water circulation in retina is affected. And then, the retina will be subjected to an ROS-producing ischemia-reperfusion situation. Based on this theory, Oharazawa et al.⁴³ observed the effects of dripping HS into the eyes with retina injured by the ischemic-reperfusion and obtained the result showing clearly that ROS production and retinal cell death were reduced.

Transplantation

Organ transplantation is a typical situation in which the phenomenon of ischemic-reperfusion occurs. If the organ is removed from the donor, it becomes ischemic at that

moment and if it is transplanted to the recipient, the recipient's blood enters it, getting into the state of reperfusion. Thus, ROS are produced in the transplant organ, which is then damaged. Therefore, the treatment of transplant organs with hydrogenated water is expected to greatly help transplanted organs survive. To prove this, Cardinal et al.⁴⁴ performed kidney transplants while administering HW to the recipients and found that inflammatory reactions, cell death, and tissue damage were reduced in the transplanted kidney. The effect of hydrogen was also observed in transplants of intestine,⁴⁵ liver,⁴⁶ heart,⁴⁷ and lung.³⁶ From these reports, the use of hydrogen is strongly recommended in organ transplants.

Aging

ROS are also known to be involved in the aging process.⁴⁸ Therefore, it can be expected that hydrogen is presumably inhibit aging. The definite way to verify the effectiveness of hydrogen on aging is to test hydrogen on humans. But it is practically impossible since there are too many markers for aging and it takes too much time. For this reason, aging studies often use insects with short life spans or animal models of aging. A typical example of the latter is the senescence accelerated mouse (SAM); the SAM dies early (normal mouse life span is 12 to 18 months, SAM is 9 months) due to premature aging process without any particular diseases⁴⁹ and reported to have a defect in the defense against ROS.⁵⁰ Gu et al.⁵¹ raised SAM while administering HW monitored hydrogen's anti-aging effects and observed that the cognitive degradation was inhibited in the SAM raised with HW.

Generalized inflammation and sepsis

A representative disease involving ROS is inflammation. So, researchers tried to check the effectiveness of hydrogen in systemic inflammation. To test the effect of hydrogen on systemic inflammation, Xie et al.⁵² injected a zymosan (a substance that stimulates white blood cells to produce ROS to provoke inflammatory reaction) to mice to induce generalized inflammation and administered hydrogen by inhalation. They found that hydrogen inhalation inhibited inflammatory cytokine production and organ damage, and increased antioxidant activity.

The most dangerous systemic inflammation is sepsis. Germs spread through the blood, and therefore enter all organs, causing functional degradation in all organs, which is called multiple organ failure. The fatality rate is close to 100% because of no specific treatment. Xie et al.⁵³ induced sepsis in mice by perforating the appendix, causing intestinal bacteria to enter the abdominal cavity and inhaled hydrogen into the septic mice. They found the decrease in organ damage and pro-inflammatory cytokine production, and increase in survival rate, which are signs indicative of definite therapeutic effects.

Metabolic syndrome and diabetes

Obesity can cause metabolic disorders and diabetes. Diabetes caused by obesity is called type 2 diabetes. The reason why obesity causes diabetes is not yet clear, but the most likely explanation is due to becoming an insulin-resistant state where insulin cannot function. Fat tissues in obesity are said to be inflamed where pro-inflammatory mediators increase and anti-inflammatory mediators decrease. The inflammation mediators interfere with insulin action thus, leading to the insulin-resistant state.⁵⁴ Based on the implication that ROS is involved in the insulin-resistance, Wang et al.⁵⁵ observed effects of HS on genetically obese rats which quickly fall into obesity and ultimately diabetes and found reduced oxidative state, weight loss, and decreased sugar and neutral fat in the blood. In another experiment⁵⁶ where HS was given to high-fat diet-fed hamsters, decreased plasma low-density lipoprotein

cholesterol levels and improved high-density lipoprotein function were observed. These results suggest the use of hydrogen is expected to be beneficial in obesity or diabetes.

Effectiveness of hydrogen in clinical studies

Following cell and animal experiments, hydrogen was observed to be effective in the clinical studies. The following are the results on human diseases.

Impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM)

IGT refers to a state in which blood sugar is higher than normal when ingested with the same amount of glucose. Although the subject with IGT does not have any symptoms of diabetes yet, it means a person who will soon become T2DM if he does not take care of his blood sugar-reducing ability. Kajiyama et al.⁵⁷ studied effects of hydrogen on the subjects of IGT and T2DM (since both are under oxidative stress due to increased ROS production) by a randomized, double-blind, placebo-controlled, crossover study in 30 patients with T2DM controlled by diet and exercise therapy and 6 patients with IGT. The patients consumed either 900 mL/day of HW or 900 mL of placebo water (PW) for 8 weeks, with a 12-week washout period. Intake of HW was associated with significant decreases in the levels of modified low-density lipoprotein (LDL) cholesterol, small dense LDL, and urinary 8-isoprostanes, and also associated with a trend of increased plasma levels of adiponectin and extracellular-superoxide dismutase (SOD). These results suggest that supplementation with HW may have a beneficial role in prevention of T2DM and insulin resistance.

Nakao et al.⁵⁸ observed the effects of hydrogen on the subjects who had one or more of the following conditions: 1) BMI, 25.0–34.9 kg/m²; 2) waist circumference, higher than 100 cm; fasting plasma glucose, 5.2–6.9 mmol/L; prehypertension (systolic: 139 mmHg or lower, diastolic: 80–89 mmHg). Subjects were asked to drink 300–400 mL of this HW (0.55–0.65 mM: 1.1–1.3 ppm) five times a day (early morning, before breakfast, before lunch, after lunch and before bedtime) for 8 weeks. In this experiment, HW was produced by placing a metallic magnesium stick into drinking water by the following chemical reaction: $\text{Mg} + 2\text{H}_2\text{O} \rightarrow \text{Mg}(\text{OH})_2 + \text{H}_2$. The results were as follows: increase in SOD and decrease in TBARS (thiobarbituric acid reactive substance) in urine, and increase in high-density lipoprotein (HDL)-cholesterol and decrease in total cholesterol/HDL-cholesterol in blood. These results suggest that drinking of HW is a safe, easy and preventive strategy for metabolic syndrome.

So far, all studies have used hydrogen-melted water to monitor the effectiveness of hydrogen. However, some drugs have been reported to increase the concentration of hydrogen in our bodies, which are α -glucosidase inhibitors (acarbose and voglibose). These drugs are known to inhibit the digestion of carbohydrates in the intestines, thus inhibiting postprandial hyperglycemia, that is known as a trigger of cardiovascular diseases. When patients with IGT were given 100 mg acarbose three times a day for about 3 years, reduction in the development of cardiovascular diseases such as hypertension and myocardial infarction were observed.⁵⁹ When voglibose (0.9 mg) was given to obese T2DM patients once a day for 3 weeks, postprandial hyperglycemia and lipidemia, and soluble intercellular adhesion molecule 1 in blood and urinary excretion of 8-isoprostaglandin F₂ α and 8-hydrodeoxyguanosine were significantly reduced.⁶⁰ The results obtained by the two groups suggest that reduction of cardiovascular complication by α -glucosidase inhibitors is due to lowering oxidative stress.

Flatulence, the major side effect of treatment of α -glucosidase inhibitors, occurs, which is due to fermentation of undigested carbohydrate by colonic bacteria, resulting in gas

formation. Suzuki et al.⁶¹ assumed that the H₂ gas is formed during the fermentation in the colon and distributes to the body. If this is true, the beneficial effects of α -glucosidase inhibitors on cardiovascular effects in patients with IGT and T2DM is due to antioxidant action of H₂. To prove this assumption, they analyzed the exhaled gas from the volunteers who take acarbose of 300 mg a day (100 mg three times a day) for 4 days and found that H₂ was increased 70–90 ppm, which is higher than about 60 ppm that obtained after drinking 900 mL of 1.2 ppm hydrogen.⁵⁷ Thus, Suzuki et al.⁶¹ strongly suggested that the beneficial effects of α -glucosidase inhibitors on cardiovascular diseases is due to formation of H₂ rather than the decreased postprandial hyperglycemia and hyperlipidemia.

Patients receiving dialysis treatment

Patients who receive dialysis treatment for a long period of time complain of various side effects, including increased blood pressure and inflammation. Hydrogen was tested on these side effects by using dialysate containing hydrogen (0.45 ppm) three times a week for 6 months.⁶² The results had a significant decrease in systolic hypertension (< 140 mmHg), and in the level of plasma monocyte attractant protein 1 and myeloperoxidase but with no side effects observed.

Acute cerebral infarct

As already explained, acute cerebral infarction is a typical ischemic-reperfusion disease. Therefore, ROS are deeply involved in brain tissue damage. Based on this, a drug called edaravone, a scavenger of the most toxic $\cdot\text{OH}$ has been for the treatment of stroke. Since hydrogen was also found to be a scavenger of $\cdot\text{OH}$,⁴ hydrogen was also tried together with Edaravone to the stroke patients.⁶³ All of the patients were brought in within 4 to 24 hours after the onset of the symptoms. One group was treated with 30 mg of edaravone (intravenous) twice a day (E group) and the other group with edaravone together with HS (300 mL of 0.5 ppm hydrogen) twice a day for 7 days (EH group). In magnetic resonance imaging (MRI), there was a therapeutic effect on MRI in both E group and EH group, but was better in EH group. Based on this result, the authors suggested the hydrogen is helpful for the treatment of this acute cerebral disorder.

Inflammatory muscular disorders

Ito et al.⁶⁴ tested the effect of hydrogen on muscular inflammatory disorders. They performed open-label trial of drinking 1.0 L/day of HW for 12 weeks in 5 patients with progressive muscular dystrophy (PMD), 4 patients with polymyositis/dermatomyositis (PM/DM), and 5 patients with mitochondrial myopathies (MM), and measured 18 serum parameters as well as urinary 8-isoprostane every 4 weeks. After open-label trial, they conducted randomized, double-blind, placebo-controlled, crossover trial of 0.5 L/day of HW or PW for 8 weeks in 10 patients with DM and 12 patients with MM, and measured 18 serum parameters every 4 weeks.

In the open-label trial, no objective improvement or worsening of clinical symptoms was observed. However, significant effects in lactate-to-pyruvate ratios in PMD and MM, fasting blood glucose in PMD, serum matrix metalloproteinase-3 (MMP3) in PM/DM, and serum triglycerides in PM/DM. were observed in HW group. In the double-blind trial, no objective clinical effects were observed, but a significant improvement was detected in lactate in MM as well as lactate-to-pyruvate ratios in MM and MMP3 in DM. HW improves mitochondrial dysfunction in MM and inflammatory processes in PM/DM. Less prominent effects with the double-blind trial compared to the open-label trial were likely due to a lower amount of administered hydrogen and a shorter observation period, which implies a threshold effect or a dose-response effect of hydrogen.

Patients receiving radiation therapy

Cancer patients who receive radiation therapy suffer from severe deterioration in their quality of life (QOL) due to various side effects, including fatigue. However, it is well known that these side effects are associated with ROS produced by irradiation.⁶⁵ To assess the effect of hydrogen on the side effects of radiation therapy, a randomized, placebo-controlled study was performed on 49 patients receiving radiotherapy for malignant liver tumors and the subjects were asked to consume HW (0.55–0.65 mM) for 6 weeks.⁶⁶ The consumption of HW reduced reactive oxygen metabolites in the blood and improved QOL scores during radiotherapy in HW group, compared to PW group. The researchers suggest that daily consumption of hydrogen-rich water is a potentially novel, therapeutic strategy for improving QOL after radiation exposure.

Skin wrinkles

Skin wrinkle is a representative sign of aging. Kato et al.⁶⁷ performed the experiments to assess the effect of hydrogen on wrinkles of human skin. Five healthy females (age: 14–65) and one healthy male (age: 55) participated in this study. The subjects took a bath filled with HW (0.19–0.41 ppm, 41°C) daily for more than 5 minutes at home for three months. The wrinkles in the back of the neck were observed by a silicon replica. On 4 subjects, the wrinkles were improved significantly. The results imply that continuous application of HW reduces oxidative stress in the skin and prevents the wrinkle formation. Thus, HW bathing could be applicable for anti-skin aging. They also confirmed the anti-aging effect of HW in the UVA-irradiated fibroblasts (OUMS-36) and keratinocytes (HaCaT). The results were as follows: Type-I collagen in fibroblasts was synthesized 1.85- to 2.03-fold by HW application for 3–5 days more than by PW, and localized preferentially around the nuclei as shown by immunostaining. HW application in ultraviolet A (UVA)-irradiated keratinocytes significantly prevented cell death and DNA damages such as nuclear condensation and fragmentation. HW significantly suppressed UVA-induced generation of intracellular $\cdot\text{O}_2^-$ in both cell lines.

Post-exercise fatigue

To produce the energy required for exercise, it causes an increase in oxygen consumption a lot. An increase in oxygen consumption is inevitably accompanied by an increase in the production of ROS. For this reason, excessive exercise is thought to be harmful. Aoki et al.⁶⁸ evaluated the effectiveness of hydrogen on excessive exercise. Ten male soccer players participated in the experiment. HW was prepared by immersing a magnesium stick into the drinking water for 24 hours. The hydrogen concentration was 0.92–1.02 mM (about 2 ppm). Subjects were provided with three bottles of 500 mL HW and asked to drink one bottle at 10:00 PM of the day before the test, one bottle at 5:00 AM and one bottle at 6:20 AM on the day of examination. The subjects were requested to use a cycle ergometer at 75% maximal oxygen uptake for 30 minutes, followed by measurement of peak torque and to do 100 repetitions of maximal isokinetic knee extension. Oral intake of HW prevented an elevation of blood lactate during heavy exercise. The peak torque of PW group significantly decreased during maximal isokinetic knee extension (a sign of muscle fatigue) but the HW group did not decrease. The results suggest that HW may be suitable hydration for athletes.

Another group of researchers⁶⁹ observed the effect of HW on oxidative stress induced by running on healthy subjects (age: 45–55, male and female: 8 subjects, respectively). The subjects did not have any particular diseases and their systolic and diastolic pressure were 90–150, 60–100 mmHg. HW (0.8–1.2 ppm) was prepared by electrolysis of water using the Jivaqua, Model JV 350 manufactured by Hyunsung Vital (Seoul, Korea). After blood (2 mL)

was taken, the participants were requested to run on the treadmill at 8 km/hr for 30 minutes. Immediately after the running, blood was sampled, then the participant were requested to drink 1,000 mL of HW and then blood was sample at 30, 60 and 120 minutes. Blood samples were used for analysis for malondialdehyde (MDA), 8-oxo-2'-deoxyguanosine (8-oxodG) and lactate. The blood levels of 8-oxodG were significantly increased after exercise. In the HW group, its levels at each time were significantly lower than those in the PW group. The effect of HW was more pronounced in females than in males. The results obtained with MDA were quite similar to those with 8-oxodG. The L-lactate levels were not significantly different statistically in both groups. The data suggest that the one time drinking of HW may suppress the exercise-induced oxidative stress.

Rheumatoid arthritis

Ishibashi et al.⁷⁰ tested the effectiveness of hydrogen on patients of rheumatoid arthritis. Twenty patients with rheumatoid arthritis (RA) drank 530 mL of HW containing 4 to 5 ppm molecular hydrogen every day for 4 weeks. After a 4-week wash-out period, the patients drank the HW for another 4 weeks. Urinary 8-hydroxydeoxyguanosine (8-OHdG) and C-reactive protein (CRP) levels was estimated at the end of each 4-week period. Drinking HW raised the concentration of H₂ more than the 1.6 ppm (saturated level in water) in vivo. Urinary 8-OHdG was significantly reduced by 14.3% ($P < 0.01$) and CRP also decreased from 3.83 to 3.02 ($P < 0.01$) during the same period. After the wash-out period, both the urinary 8-OHdG and the mean CRP decreased, compared to the end of the drinking period. During the second drinking period, the mean CRP was reduced while urinary 8-OHdG was not reduced further but remained below the baseline value. All the 5 patients, 4 of them became symptom-free at the end of the study.

Erythematous skin diseases

Ono et al.⁷¹ tried hydrogen on the patients of acute erythematous skin diseases. All of the four patients received intravenous administration of 500 mL of HW in 30 minutes for more than 3 days except in one patient for only once. Erythema of these 4 patients and associated symptoms improved significantly after the H₂ treatment and did not recur. Administration of HW did not cause deterioration of the blood chemistry. From two volunteers (one for intravenous HW administration and the other for H₂ inhalation), blood samples were withdrawn serially and air samples were collected from a heavy duty plastic bag covering a leg, before, during and after H₂ administration. These samples were checked for H₂ concentration immediately by gas chromatography. The H₂ concentration in the blood from the volunteers rapidly increased with H₂ inhalation and slowly decreased with cessation of H₂ particularly in the venous blood, while H₂ concentration of the air from the surface of the leg showed much slower changes even after H₂ inhalation was discontinued, at least during the time of sample collection.

METHODS TO ADMINISTER HYDROGEN

Since 2007 when Ohsawa et al.⁴ reported hydrogen removed the most toxic •OH of ROS and inhibited brain tissue damage caused by ischemic-reperfusion in the rat brains, numerous researchers have been performing lots of experiments in animals and humans to demonstrate the effectiveness of hydrogen aiming at using hydrogen as a therapeutic use for many diseases. Here, how hydrogen is applied to various experiments is summarized.

Inhalation of hydrogen gas

Inhalation of hydrogen gas is a straightforward therapeutic method. Since the inhaled gas can diffuse rapidly into the tissues, it is recommended for defense against the condition of acute oxidative stress in the brain and heart. In most cases, 2% hydrogen gas was found to be effective^{4,26,72} without the increase of blood pressure,⁴ which may cause serious obstacles during the treatment of myocardial infarction. Hydrogen gas at concentration less than 4% does not explode in air and in pure oxygen. The diffusion of inhaled hydrogen gas into the blood, and the normal and pathologic tissues was proven by monitoring its concentration inside the blood and tissues.²⁶

Oral administration of HW

Inhalation has the advantage of being quick to work, but it is inconvenient for the general public to use it daily for a long time. So, the easy way to use it is to drink HW. The maximum concentration obtained by passing the hydrogen gas through the water is 1.6 ppm.³⁵ ppm means the number of grams of a substance in 1,000,000 mL (1,000 L) of water thus, 1.6 ppm of hydrogen concentration indicates 1.6 g of hydrogen in 1,000 L of water. However, in most animal or human experiments, the concentration of hydrogen used ranges from a minimum of 0.4 ppm to a maximum of 1.2 ppm. One thing to note about the use of HW is that it can easily escape away from the container. To prevent this, HW is put in an aluminum bag (hydrogen is easily melted into metals, but it does not melt into aluminum) with tightening the cap. Even in the aluminum container, hydrogen concentration decreases in half after 24 hours. Therefore, if you put an aluminum bag containing 1.2 ppm of hydrogen to the animal cage, the concentration of hydrogen decreases over time and the hydrogen concentration animals drink becomes 1.0 ppm or less. Surprisingly, the effect of drinking HW is comparable to that of inhaling hydrogen. So long-term use of HW is a very convenient way of hydrogen administration. Hydrogen in water tends to evaporate in the gastrointestinal tract. To prevent this, hydrogen is used to dissolve hydrogen in saline (0.9% NaCl) instead of water. HS can be used for direct application to the affected areas or for injection to vessels and abdominal cavity.^{14,18,22}

Direct administration to the affected area

In case of eye diseases such as glaucoma or retinal diseases, HS is used by administering directly to the eyes. In addition to the eyes, direct administration can be used to the exposed affected areas anywhere. When drops of HS were applied to the eye continuously, H₂ concentration in the vitreous body started to increase after 2 min, and reach a maximum level after 15 minutes. This level is about 20% of the eye drops of HS.⁴³

HW bath

Hydrogen easily penetrates the skin, enters the blood and distributes throughout the body. Warm bath in HW is also a good way to administer hydrogen to the whole body.⁶⁷ Hydrogen is detected in the exhaled air in 10 minutes after taking a bath, indicating how fast hydrogen penetrates the skin.⁷³

Method to promote hydrogen production in the intestine

As mentioned earlier, in some experiments, effects of hydrogen was observed by promoting production in the intestine, not by inhaling or drinking hydrogen directly through the nose or mouth. Humans cannot produce hydrogen on their own because there are no enzymes to produce hydrogen. However, hydrogen is produced from the carbohydrates that are not digested in the intestines when fermented by intestinal bacteria. α -Glucosidase inhibitors

suppress carbohydrate digestion, resulting in accumulation of undigested carbohydrates in the intestines thus, inhibiting postprandial hyperglycemia after meals. It was reported that the use of these drugs for the treatment of type 2 diabetes significantly reduced the incidence of cardiovascular diseases.⁵⁹ However, insulin or oral hypoglycemic agents can lower blood sugar levels, but they are not very effective in preventing cardiovascular complications. α -Glucosidase inhibitors induce production of hydrogen in the intestines⁶¹ while insulin or oral hypoglycemic agents do not. Therefore, the decrease of cardiovascular complication by α -glucosidase inhibitors is thought to be ascribed to the reduction of oxidative stress by hydrogen produced in the intestine, which distributes to all parts of the body. Consistent with these findings, it was observed that suppression of intestinal bacterial flora by antibiotics significantly decreased the level of H_2 in both liver and intestine and increased the severity of concanavalin A-induced hepatitis.³¹

ADVANTAGE OF HYDROGEN COMPARED TO OTHER ANTIOXIDANTS

Hydrogen is emerging as an antioxidant that is effective in preventing and treating diseases associated with ROS as it has characteristics different from those of conventional antioxidants. Its characteristics are summarized as follows:

First, hydrogen is characterized by scavenging only most reactive $\bullet OH$, not $\bullet O_2^-$, and H_2O_2 .⁴ Of the three species, tissues have no enzyme to remove $\bullet OH$. Compared to $\bullet OH$, $\bullet O_2^-$ and H_2O_2 are less reactive, and these species, at lower concentrations, are involved in cell signaling rather than cell destruction, serving as signaling mediators in major cellular functions such as cell proliferation and differentiation. Therefore, their removal can interfere with cell functions.⁷⁴ Thus, hydrogen has the advantage of being able to suppress oxidative stress very efficiently without disrupting cell functions because it only removes the most toxic $\bullet OH$ and does not remove $\bullet O_2^-$ and H_2O_2 .

Second, hydrogen diffuses throughout the body very quickly because it can pass through the lipid cell membrane very easily.²⁶ Therefore, it has the advantage of being able to be quickly delivered to the sites of lesion. More importantly, hydrogen easily enters mitochondria across the mitochondrial membranes, which is a major site of ROS production. Thus, hydrogen immediately and efficiently removes $\bullet OH$ from the site of production source.⁴ By the same token, hydrogen can get into the nucleus and protect genetic materials in the nucleus from the attack of $\bullet OH$. Many existing antioxidants have the limitation in the treatment of diseases probably because they cannot pass through cell membranes as easily as hydrogen does.^{75,76}

Because hydrogen can easily pass through the cell membrane, it can also easily pass through the blood-brain barrier (BBB) and enter the brain tissue quickly. The blood vessels in the brain tissue are surrounded by special devices, making it impossible for substances in the blood vessels to enter the brain tissue easily. This is to protect the brain. However, when a brain disease occurs and you want to treat it with a drug, it is difficult for the drug administered to pass through the BBB, which makes it difficult to treat brain lesion. Fortunately, hydrogen can easily reach brain tissue without being interrupted by BBB.

Third, hydrogen is not toxic at high concentrations. Its nontoxicity is being proved in deep-sea divers.⁷⁷ In deep-sea diving, a large amount of gas (mainly nitrogen) is dissolved in the

blood by water pressure (gases melt well in water under high pressure). This causes divers to fall into a coma, a state anesthetized by nitrogen, thus, it is called nitrogen narcosis. In addition, when divers come to the surface after diving, the dissolved gas is released from the blood as bubbles due to pressure lowering. If the bubbles block blood vessels entering major organs such as the brain and heart, divers will be in an emergency situation. This is called decompression sickness. In order to treat and prevent the dangerous nitrogenous coma and decompression sickness, the diver is asked to drink a special mixture of 49% hydrogen, 50% helium and 1% oxygen. Even in this high concentration of hydrogen, there are no side effects or toxic effects caused by hydrogen. For this reason, 49% hydrogen is already recognized as a 'safety standard for use.' In animal and human experiments, hydrogen mixture with air (2%) or HW up to 1.6 ppm (1.6 g of hydrogen per 1,000 L of water) were used. These concentrations are very low compared to 49%. Therefore, there is no need to worry about toxicity in the amount of hydrogen currently being used for the diseases.

CONCLUSION

Hydrogen has been the focus of industrial interest as a pollution-free fuel. Moreover, hydrogen is also a hot topic in medicine as ROS scavenger.

Normally, production and removal of ROS are equilibrated in the body. Sometimes, however, this equilibrium is shifted to accumulation of ROS, giving harmful effects to the body, which is called oxidative stress. Now, it is revealed that almost all diseases that plague us are directly or indirectly related to oxidative stress. Therefore, effective elimination of ROS can greatly help treat and prevent diseases.

Recently, hydrogen has been found to be antioxidant. Through many experiments on animals and humans, hydrogen has been found to be effective on diseases involving ROS. Moreover, hydrogen has many advantages over conventional antioxidants, which are: eliminating specifically only $\bullet\text{OH}$ (the most toxic active oxygen), nontoxic, absorbed rapidly to the cells, and penetrating all parts of the body.

Many efforts have been made to develop hydrogen as a therapeutic. Before hydrogen being a drug, however, it will help us stay healthy by eliminating the threat of ROS if normal healthy persons inhale hydrogen gas or drink HW, like taking vitamins every day.

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